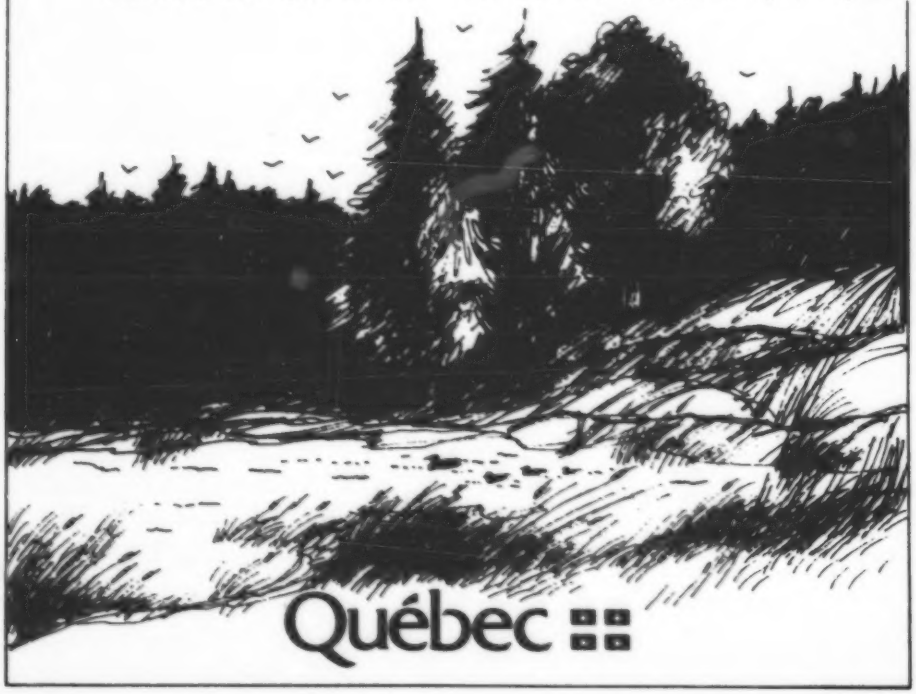
CONCENTRATIONS OF BIOLOGICAL INSECTICIDES IN THE AIR OF CERTAIN MUNICIPALITIES FOLLOWING AERIAL SPRAYING AGAINST THE SPRUCE BUDWORM IN 1987



Québec

88-05731/

CONCENTRATIONS OF BIOLOGICAL INSECTICIDES IN THE AIR OF CERTAIN MUNICIPALITIES FOLLOWING AERIAL SPRAYING AGAINST THE SPRUCE BUDWORM IN 1987

Jacques Dugal, Biologist-Planner

Service des études environnementales

Gouvernement du Québec
Ministère de l'Énergie et des Ressources
Direction de la conservation

Québec City, August 1988

87a

ACKNOWLEDGEMENTS

The authors wish to express their heartfelt appreciation to Ann Carey of the Air Monitoring Branch (EPA) and Ernest Richardson of the Maine Department of Human Services for having loaned us the samplers necessary for this study. We would also like to thank the contract employees--Francine Hovington, Françoise Gendron, Adrienne Ouellet and Daniel Charon--who carried out the sampling. Finally, we would like to extend our thanks to the staff of the Service des études environnementales who contributed to the realization of this study:

Marie-France Bérubé, Secretary
Céline Blanchet, Laboratory Technician
Pierrete Cardinal, Microbiologist
Luc Major, Biologist
Hélène Marquis, Technician, Environment
Guy Parent, Wildlife Technician
Ghislain Rousseau, Technician, Environment

Note: This document was revised by Gilles Gaboury and Luc Major. Microbiologist Jean Cabana also served as reviser, particularly as concerns the information contained in section 4.2.

ABSTRACT

In 1987, the ministère de l'Énergie et des Ressources carried out its eighteenth operation to combat the spruce budworm (Choristoneura fumiferana, Clem.). In accordance with its environmental monitoring program, the Service des études environnementales conducted a study to determine the drifting of the biological insecticide Bacillus thuringiensis (B.t.) into four municipalities near the treated areas. A total of 159 air samples were taken in the municipalities of Colombier, Manche-d'Épée, Sainte-Florence and Saint-François-d'Assise between May 15 and June 25, 1987. These samples showed concentrations ranging from non-detectable to 59.54 C.F.U./m^3; less than 5% of the samples contained concentrations higher than 1 C.F.U./m^3. If these results are compared with those of previous years, it will be seen that the concentrations of B.t. in municipalities near treated areas have diminished since 1984. A number of factors might be responsible for this: the decrease in the size of areas treated, changes in the composition of the commercial preparation, changes in the type of aircraft used, or meteorological factors.

In conjunction with this sampling program, 12 additional samples were taken in Manche-d'Épée to determine the level of contamination due to bacteria of the type Enterococcus sp.; these bacteria were present in commercial preparations used in 1987. Low concentrations of 0.05 C.F.U./m^3 and 0.2 C.F.U./m^3 were detected on two of the twelve sampling days. Enterococcus faecium was identified as being responsible for this low-level contamination.

The concentrations of B.t. and enterococci detected during the study are not likely to represent a health hazard for the populations of these municipalities.

SOMMAIRE

Le ministère de l'Énergie et des Ressources a réalisé en 1987 une dix-huitième opération contre la tordeuse des bourgeons de l'épinette (Choristoneura fumiferana, Clem.). Le Service des études environnementales du ministère a effectué, conformément à son programme de suivi environnemental, une étude visant à évaluer la dérive de l'insecticide biologique Bacillus thuringiensis (B.t.) dans quatre municipalités localisées à proximité des secteurs traités. Au total, 159 échantillons d'air ont été prélevés entre le 15 mai et le 25 juin 1987 dans les municipalités de Colombier, Manche-d'Épée, Sainte-Florence et Saint-François-d'Assise. L'analyse des échantillons a révélé des concentrations variant de non-détectées à 59.54 U.F.C./m^3 et que seulement 5% des échantillons contiennent des concentrations supérieures à 1 U.F.C./m^3. Comparativement aux résultats obtenus lors des années précédentes, on constate que les concentrations de B.t. dans les municipalités avoisinantes des aires traitées diminuent depuis 1984. Des facteurs tels que la diminution des superficies traitées, les facteurs météorologiques, le type d'aéronefs utilisés de même que la composition de la préparation commerciale pourraient influencer le comportement de l'insecticide dans l'air et ainsi expliquer la différence observée entre les années.

Parallèlement à ce programme d'échantillonage, 12 échantillons supplémentaires ont été prélevés à Manche-d'Épée dans le but d'évaluer le niveau de contamination provoqué par des bactéries de type Enterococcus sp. présentes dans les préparations commerciales utilisées en 1987. De faibles concentrations de 0.05 et 0.2 U.F.C./m^3 ont été détectées pendant deux des douze journées de prélèvement. L'espèce Enterococcus faecium a été identifiée comme étant responsable de cette faible contamination.

Les concentrations de B.t. et d'entérocoques détectées au cours de cette étude ne seraient pas susceptibles de répresenter un risque pour la santé des citoyens des municipalités concernées.

TABLE OF CONTENTS

ACKNOWLEDGEMENTS iii

ABSTRACT v

SOMMAIRE vii

TABLE OF CONTENTS ix

INTRODUCTION 1

1. TERRITORY 3

2. METHODOLOGY 5

2.1 Spraying 5
2.2 Insecticide preparation 5
2.3 Location of sampling stations 6
2.4 Air sampling 6

2.4.1 Bacillus thuringiensis 6
2.4.2 Enterococcus sp. 8

2.5 Analytical method 8

2.5.1 Bacillus thuringiensis 8
2.5.2 Enterococcus sp. 10

3. RESULTS 11

3.1 Bacillus thuringiensis 11

3.1.1 Colombier 11
3.1.2 Manche-d'Epée 11
3.1.3 Sainte-Florence 11
3.1.4 Saint-François-d'Assise 11

3.2 Enterococcus sp. 13

4. DISCUSSION 15

4.1 Bacillus thuringiensis 15
4.2 Enterococcus sp. 17

BIBLIOGRAPHY 19

APPENDIX 1: Spraying operations carried out by the MER in 1987 21

APPENDIX 2: Composition of nutrient agar, Tween 80 and of M. Enterococcus selective culture medium 22

TABLE 1: Concentrations of biological insecticide (B.t.) detected in air samples taken in municipalities surveyed in 1987 12

FIGURE 1: Spruce budworm 1987 program - areas treated 4

FIGURE 2: Diagram of apparatus used in sampling air for biological insecticides in 1987 7

INTRODUCTION

In the spring of 1987, as part of its program to combat harmful insects in the forest environment and in accordance with the Order in Council 192-85, le ministère de l'Énergie et des Ressources carried out its 18th consecutive spraying program against spruce budworm (Choristoneura fumiferana, Clem.). The purpose of the operation, consisting of the aerial spraying of biological insecticides, was to reduce damage caused by this insect, by reducing larvae populations below the level which is considered economically harmful.

The Service des études environnementales (SEE) of the Ministère carried out a study to evaluate insecticide drifting into certain municipalities located close to the areas treated. This study, which came within the scope of the environmental monitoring program related to the spraying of insecticides in the forest environment (Dostie et al., 1987), enabled the researchers to measure the concentrations to which the citizens of these municipalities may be exposed. Similar studies have been conducted in recent years, notably with chemical insecticides (Major et al., 1984a; 1984b; 1986a; Dugal and Major, 1987) and biological insecticides (Major et al., 1985, 1986b; Dugal and Major, 1987).

In 1987, during quality control of the insecticides, the microbiology laboratory of the SEE identified the presence of Enterococcus sp. in the commercial preparations of B.t. used in Québec to combat spruce budworm. For this reason we began another program of air sampling in order to evaluate the level of contamination caused by these bacteria in one of the municipalities in question. It should be noted, however, that the contamination of the lots of insecticide by these microorganisms was judge to have little importance, given their nature and the low concentrations present in the insecticide.

1. TERRITORY

A surface area of 197 992 hectares divided between the Côte-Nord and Bas-St-Laurent-Gaspésie regions was treated during the 1987 spraying program (Figure 1). It is important to note that spraying operations were also carried out in private forests by the Canadian Forestry Service (CFS). These operations, which also used biological insecticide, covered a total surface area of 7 100 hectares (Anonymous, 1987). The areas treated by the CFS do not appear in Figure 1 as the blocks involved were too small. However, since they were situated in the same general area and often in close proximity to the sampling stations, they were taken into account in the interpretation of the results.

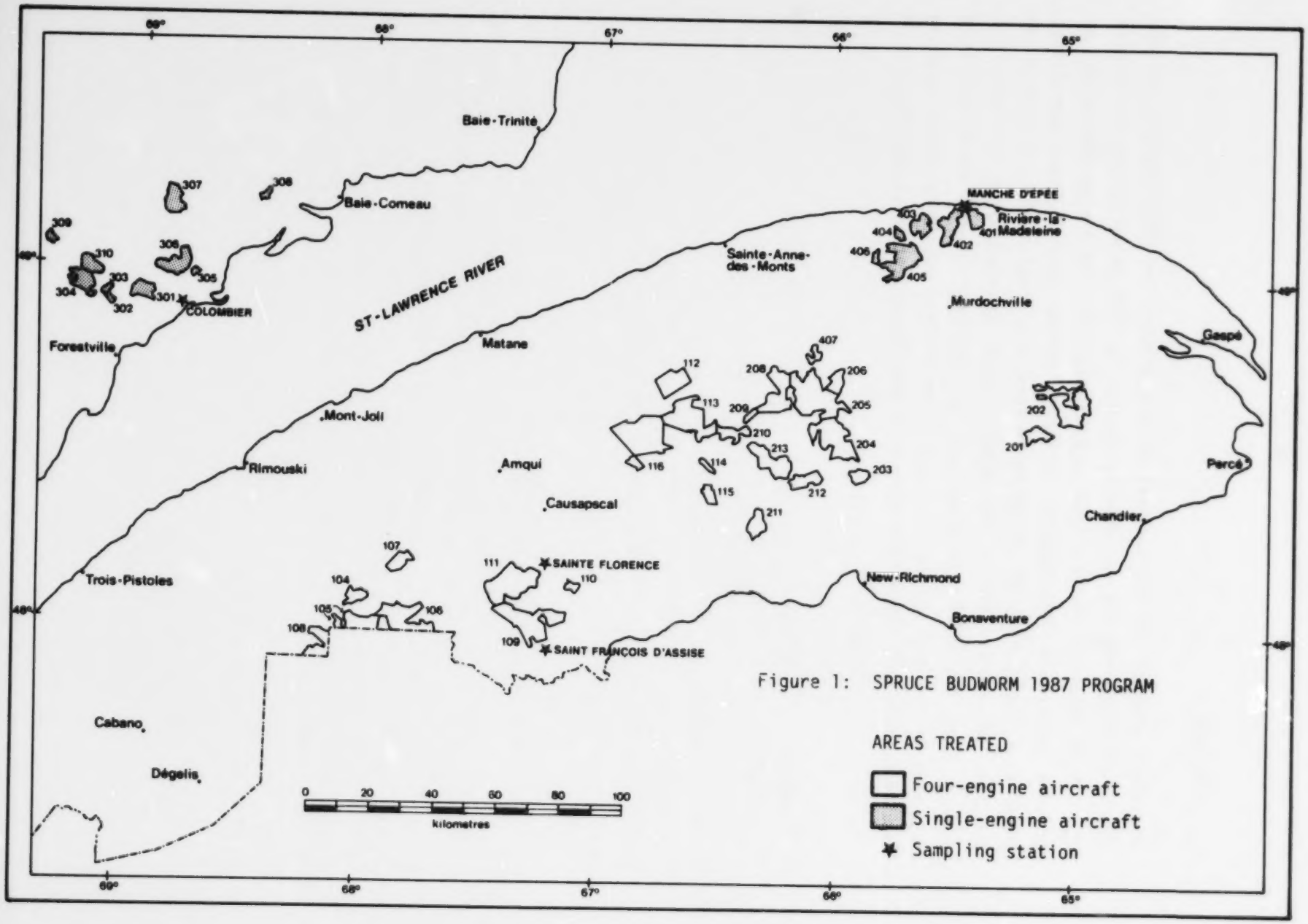


Figure 1: SPRUCE BUDWORM 1987 PROGRAM

2. METHODOLOGY

2.1 Spraying

The spraying program was carried out from May 31 to June 19, 1987 (Appendix 1). The equipment used during this operation consisted of DC-4 and DC-6 four-engine aircraft, as well as Turbo Thrush and Bull Thrush single-engine aircraft. The four-engine aircraft, guided by Litton^{tm} inertial navigation systems, were equipped with a spraying system consisting of booms fitted with 110 Spraying System^{tm} open nozzles. The spraying system installed on the single-engine aircraft consisted of booms fitted with 6 Mini-Micronair^{tm} AU-5000 atomizers. During the spraying operations, the single-engine aircraft were navigated visually with the help of a guide plane. It should be noted that the blocks treated received only one application of insecticide.

2.2 Insecticide preparation

For the first time in Québec, only biological insecticide was used to combat the spruce budworm. Dipel^{tm} 132, of which the active ingredient is the Bacillus thuringiensis var. kurstaki, was used operationally on 197 065 hectares of public forest. This product was sprayed at a rate of 30 BIU[1]/ha for a final volume of 2.37 ℓ/ha. An experimental block with a surface area of 927 hectares was sprayed with Dipel^{tm} 176 at a rate of 30 BIU/ha to give a final volume of 1.77 ℓ/ha.

As mentioned above, another spraying program was carried out by the Canadian Forestry Service (CFS). In this program, Futura^{tm} was sprayed over 7 100 hectares of private forest in southeastern Québec at a rate of 20 BIU/ha to give a final volume of 2.5 ℓ/ha (Anonymous, 1987).

---

1 BIU: Billions of international units.

2.3 Location of sampling stations

The municipalities of Colombier on the Côte-Nord, and Manche-d'Épée, Sainte-Florence and Saint-François-d'Assise in Gaspésie, were selected for the study (Figure 1). The sampling stations were set up in the backyards of private homes on open and relatively flat ground. These stations were located downwind from the areas treated, and less than 5 km separated the stations from the closest of these areas.

2.4 Air sampling

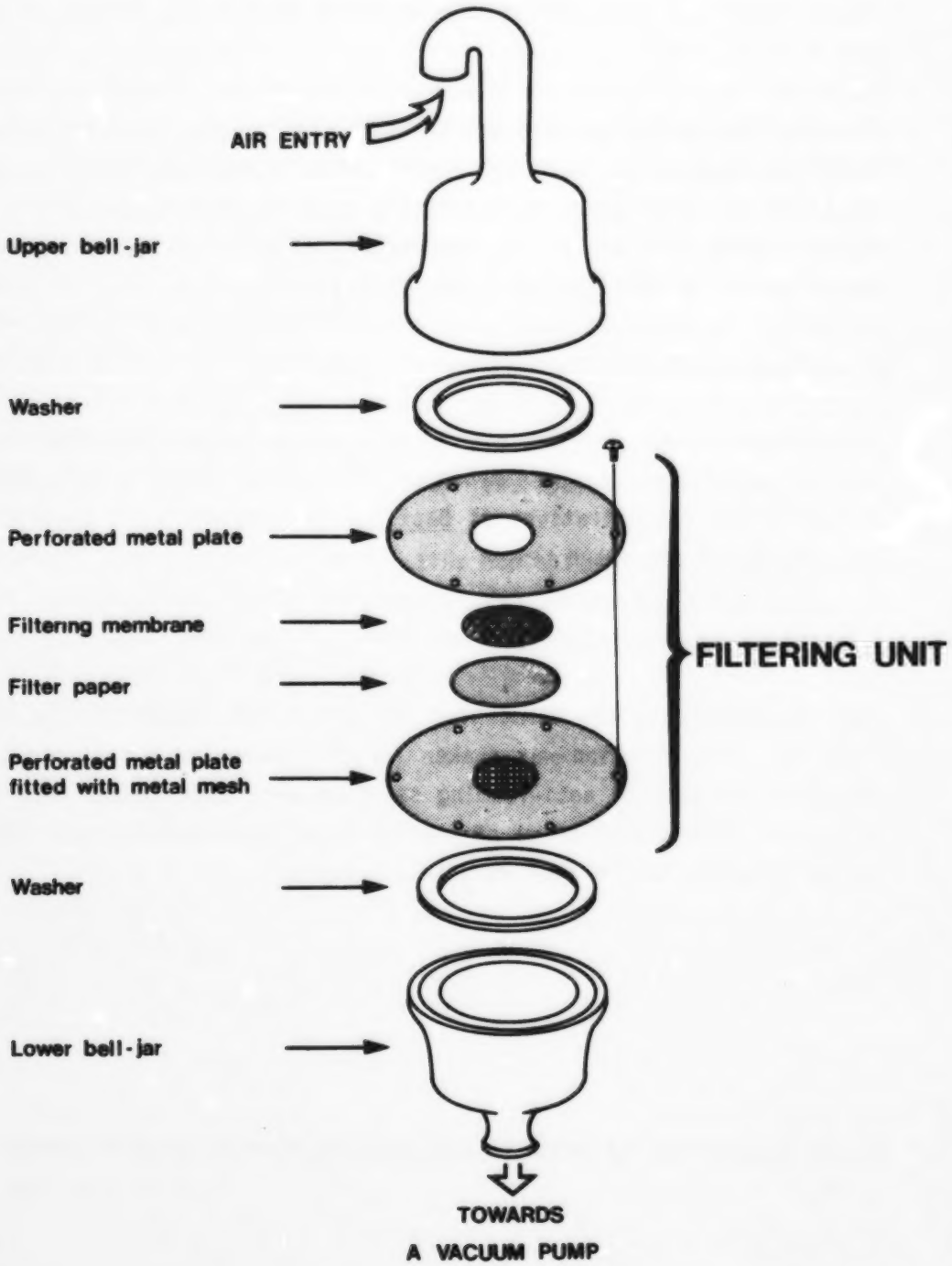
2.4.1 Bacillus thuringiensis

At each one of these stations, the air sampling program began approximately two weeks before the spraying operations, and ended approximately one week after spraying was finished. Samples were taken using a Sierra-Misco^{tm} Model 88 sampling apparatus at a flow rate of about 20 ℓ/min. Each air sampling period lasted approximately 24 hours.

The filtering unit consisted of a filtering membrane, a filter paper and two perforated metal plates. B.t. spores were retained by a Millipore^{tm} filtering membrane of cellulose ester, with a porosity of 0.45 µm and a diameter of 4.7 cm. The membrane was placed on a Whatman^{tm} filter paper between the two perforated stainless steel plates (Fig. 2). The filtering unit was sterilized with ethylene oxide under hospital conditions and remains sealed until use. It should be noted that, following laboratory tests carried out in 1987, this method of sterilization has proved to be highly efficient.

During sampling, the filtering unit was installed between two presterilized glass bell-jars. The system was sealed using rubber washers and the apparatus was then connected to a pump. Major et al. (1985) mention that this type of apparatus is highly effective in retaining B.t. spores. After samples were taken, the filtering unit was placed in a sterile Petri dish and kept in a freezer, until laboratory analysis.

Figure 2: Diagram of apparatus used in sampling air for biological insecticides in 1987.



2.4.2 Enterococcus sp.

The monitoring program designed to evaluate the levels of contamination by Enterococcus sp. was carried out in Manche-d'Épée. The sampling in this region began two days before the spraying operations, which took place from June 5 to 16, 1987.

The air sampling method used was identical to the one described above, the only exception being that, once the samples were taken, the filter was removed from the filtering unit and placed directly on an M. Enterococcus selective culture medium. This step caused the incubation period to begin. The sample was then sent directly to the laboratory for analysis.

2.5 Analytical method

The analyses were carried out in the microbiology laboratory of the MER's Service des études environnementales (SEE). The analysis methodology used to determine the concentrations of Bacillus thuringiensis and Enterococcus sp. in the samples is presented below.

2.5.1 Bacillus thuringiensis

The filtering unit was dismantled and the filter placed in 200 ml of sterile Tween 80[1]. The solution was shaken for 30 minutes on a mechanical agitator. A few drops of sterile anti-foaming solution were then added. Subsequently, the filter was rinsed with 50 ml of sterile water and placed on nutrient agar[1]; the spores adhering to the agar were later counted.

---

1 The composition of Tween 80 and nutrient agar is given in detail in Appendix 2.

The total volume of 250 ml was shaken for 30 seconds for better homogenization. Two samples of 50 ml and one of 100 ml were taken and analyzed by vacuum filtration using funnels fitted with Millipore^{tm} filters with a porosity of 0.45 µm. These filters were then placed on nutrient agar for a 24-hour incubation period at 29^{o}C.

The spores were counted by selecting Petri dishes containing between 20 and 80 colonies. Only colonies resembling B.t., that is, beige in colour, and flat with a rough surface, were taken into account. The B.t. percentage was determined on a Petri dish which had been re-incubated for a total of 50 hours. This incubation period permitted spore development and the release of crystals (protein bodies) characteristic of B.t. From this Petri dish, slide smears were prepared using 20 colonies. A stain developed by Smirnoff (1962) was applied to highlight the B.t. crystals. The remaining 50 ml sample was stored at 4^{o}C. When more than 80 B.t. colonies were counted on the filtering membrane, smaller volumes were filtered. The quantity retained by the sampling filter was added to the total number of C.F.U.[1] extrapolated for 250 ml.

All materials were handled under a laminar flux biological hood so as to reduce the risks of contamination. Reference samples from before and after filtering were analyzed to ensure that the equipment was sterile. The detailed analysis methodology is described in Cardinal (1986).

---

1 C.F.U.: Colony-forming units; these may originate from one or more spores (Collins and Lyne, 1984).

2.5.2 Enterococcus sp.

As indicated above, the sample was sent to the laboratory in a Petri dish containing an M. Enterococcus[1] selective culture medium. The sample was then incubated for approximately 48 hours at a temperature of 37°C. The identification and counting of the fecal streptococci were made possible by the reduction of triphenyltetrazolium chloride, a colourless compound, into formosan, a brick-red compound.

In order to distinguish the Enterococcus faecalis species from the Enterococcus faecium species, the red colonies were subcultured onto blood agar. After an incubation period of 18 to 24 hours at a temperature of 37°C, the colonies were suspended in a physiological serum for identification using API™ galleries.

---

1 The composition of the M. Enterococcus selective culture medium is given in Appendix 2.

3. RESULTS

3.1 *Bacillus thuringiensis*

A total of 159 air samples were taken in the four municipalities under study. The results, expressed in C.F.U./m^3, show the mean concentrations observed over a period of 24 hours (Table 1).

3.1.1 Colombier

Over the 38 sampling days at Colombier, only one sample was found to contain *B.t.*, at a concentration of 0.29 C.F.U./m^3. This sample was taken on June 10, 1987.

3.1.2 Manche-d'Épée

Between May 16 and June 25, 1987, *B.t.* was detected in 11 of the 41 samples taken at Manche-d'Épée. These very low concentrations ranged from 0.13 to 0.39 C.F.U./m^3. One sample was invalidated because of filter breakage.

3.1.3 Sainte-Florence

Only 3 of the 39 samples taken at Sainte-Florence revealed the presence of *B.t.* One sample was invalidated because of filter breakage. The concentrations were 0.15, 0.43 and 0.73 C.F.U./m^3 respectively.

3.1.4 Saint-François-d'Assise

Between may 17 and June 25, 1987, 40 air samples were taken. The concentrations of *B.t.* ranged from "undetected" (less than 0.11 C.F.U./m^3) to 59.54 C.F.U./m^3.

TABLE 1: Concentrations of biological insecticide (B.t.) detected in the air samples taken in the municipalities surveyed in 1987

DATE[1]	CONCENTRATION (C.F.U./m^3)[2]			
	COLOMBIER	MANCHE-D'ÉPEE	STE-FLORENCE	ST-FRANCOIS-D'ASSISE
87-05-15	N.D.[3]	-	-	-
16	N.D.	N.D.	-	-
17	N.D.	N.D.	N.D.	N.D.
18	N.D.	N.D.	N.D.	N.D.
19	N.D.	N.D.	N.D.	N.D.
20	N.D.	*	*	N.D.
21	N.D.	0.15	N.D.	N.D.
22	N.D.	N.D.	N.D.	N.D.
23	N.D.	N.D.	N.D.	N.D.
24	N.D.	N.D.	N.D.	N.D.
25	N.D.	N.D.	N.D.	N.D.
26	N.D.	0.14	N.D.	N.D.
27	N.D.	0.39	N.D.	N.D.
28	N.D.	N.D.	N.D.	0.22
29	N.D.	0.13	N.D.	N.D.
30	N.D.	N.D.	N.D.	N.D.
31	N.D.	N.D.	N.D.	0.30
06-01	N.D.	N.D.	N.D.	3.00
02	N.D.	N.D.	N.D.	5.19
03	N.D.	N.D.	N.D.	59.54
04	N.D.	N.D.	N.D.	5.57
05	N.D.	N.D.	N.D.	1.12
06	N.D.	N.D.	N.D.	2.70
07	N.D.	0.17	N.D.	N.D.
08	N.D.	N.D.	N.D.	0.23
09	N.D.	N.D.	N.D.	0.37
10	0.29	0.25	N.D.	0.15
11	N.D.	N.D.	N.D.	N.D.
12	N.D.	N.D.	N.D.	N.D.
13	N.D.	0.16	N.D.	0.19
14	N.D.	0.15	0.73	0.28
15	N.D.	0.14	N.D.	0.11
16	N.D.	0.15	N.D.	N.D.
17	N.D.	N.D.	N.D.	N.D.
18	N.D.	N.D.	0.15	N.D.
19	N.D.	N.D.	0.43	N.D.
20	N.D.	N.D.	N.D.	N.D.
21	N.D.	N.D.	N.D.	N.D.
22	-	N.D.	N.D.	0.14
23	-	N.D.	N.D.	1.29
24	-	N.D.	N.D.	N.D.
25	-	0.15	N.D.	N.D.

1 Date sampling period began.
2 Mean concentrations over 24 hours.
3 Not detected (less than 0.11 C.F.U./m^3 or 3 C.F.U. in sample).
* Sample invalidated.

3.2 *Enterococcus sp.*

Over the 12 sampling days at Manche-d'Épée, the presence of *Enterococcus*-genus bacteria was detected on two consecutive days, June 7 and June 8, 1987. The concentrations were 0.05 and 0.2 C.F.U./m^3 respectively (the equivalent of 1 and 4 spores in the 24-hour sample). Additional analyses showed that the species *Enterococcus faecium* was responsible for this very slight contamination.

4. DISCUSSION

4.1 Bacillus thuringiensis

In each of the four municipalities under study, the concentrations of B.t. detectable in the air (0.11 C.F.U./m^3 and over) were observed sporadically throughout the entire duration of the sampling program. However, the level of contamination was very low. Less than 5% of the samples analyzed revealed concentrations exceeding 1 C.F.U./m^3, and they were all taken in the municipality of Saint-François-d'Assise. The concentrations found at Colombier, Manche-d'Épée and Sainte-Florence did not exceed 0.75 C.F.U./m^3.

Although it is not possible to establish a direct correlation between the distance of the treated areas, the circumstances of the spraying and the concentrations measured daily in each of the municipalities, it should be noted that 6 of the 7 concentrations exceeding 1 C.F.U./m^3 were observed after spraying had been carried out less than 20 km from the sampling station. This observation corroborates the results obtained in 1986 (Dugal and Major, 1987).

Between June 1 and 6 1987, during which period the concentrations found at Saint-François-d'Assise exceeded 1 C.F.U./m^3, the MER and the CFS carried out 3 and 5 spraying operations respectively, less than 20 km from the sampling station (Appendix 1; Anonymous, 1987). This period corresponds to the period during the summer of 1987 when the spraying operations carried out in close proximity to the sampling stations were the most intensive.

A concentration of 1.29 C.F.U./m^3 was detected at Saint-François-d'Assise on June 23, 1987, even though the spraying program in this municipality had ended on June 18, 1987; neither had any B.t. treatments been carried out in the agricultural environment (Marcel Michaud, MAPAQ regional office - Rimouski, personal communiqué, 1988). This elevated level of contamination may have been caused by handling or faulty analysis.

For the sake of comparison, the results obtained in 1984, 1985 and 1986 in various municipalities of eastern Québec were considered. The percentage of the results in which the level does not exceed 1 C.F.U./m^3 for each year is,

respectively, 63%, 87% and 91% (Dugal and Major, 1987). In 1987, 95% of the concentrations were below 1 C.F.U./m^3. The maximum concentration detected in 1987 was 59.54 C.F.U./m^3, whereas levels of up to 938.24 C.F.U./m^3 (1985) had been detected in previous years (Dugal and Major, 1987). It is interesting to note that the concentrations of B.t. in the municipalities situated in proximity to the treated areas were inferior to those of previous years. Factors such as the extent of annual spraying programs, weather conditions, the type of aircraft, and the composition of the commercial preparation of the product used could have influenced the amount of insecticide which drifted from the treated area. Nevertheless, it must be kept in mind that no one of these factors can wholly account for the decrease in the concentrations of B.t. which has taken place outside the treated areas since 1984.

In 1984 and 1987, the surface areas treated with B.t. were 306 000 ha, 483 000 ha, 18 000 ha and 198 000 ha respectively. The spraying programs carried out during the last two years were therefore of lesser scope than the 1984 and 1985 programs. As far as weather conditions are concerned, wind direction and velocity, temperature and humidity are factors which tend to considerably affect the distribution of insecticide in the air. However, any attempt to establish a direct correlation between weather conditions and the concentration levels detected in the municipalities, in order to demonstrate the decrease in the concentrations observed since 1984, would be somewhat risky.

Dugal and Major (1987) point out that the exclusive use of single-engine aircraft seems to contribute to a decrease in concentrations found in the vicinity of the treated areas, given that single-engine planes operate at a lower altitude than four-engine planes. This year's results corroborate the findings of Dugal and Major: the maximum concentration detected at Colombier and Manche-d'Épée was 0.39 C.F.U./m^3, while Saint-François-d'Assise, a municipality sprayed by four-engine aircraft, revealed concentrations of up to 59.54 C.F.U./m^3.

In 1987, the commercial preparations of B.t. used in Québec contained a pharmaceutical type of mineral oil. These preparations differed from those used in previous years which were water-based. Given the fact that the rate of evapo-

ration of these two components is different, it can be expected that the behaviour of the insecticide in the air will be noticeably modified, depending on whether the product used is oil- or water-based.

The low concentrations of B.t. detected during this monitoring study are the same or lower than those detected in the studies carried out in previous years and, as such, present only a minimal hazard for the populations concerned (Major et al., 1985).

4.2 Enterococcus sp.

During the quality control of the commercial preparations of B.t. used in Québec, the microbiology laboratory of the SEE identified the presence of bacteria of the type Enterococcus faecalis and Enterococcus faecium. After consultation of specialized health organizations and of the proper authorities, the contamination of the lots of insecticide by these bacteria was judged to be minimal, given the low concentrations found and the very nature of the bacteria. Consequently, the ministère de l'Environnement authorized aerial spraying of B.t. over all of the areas comprised in the 1987 program.

In general, enterococci are accurate indicators of pollution and health conditions (Diebel and Hartman, 1976). Indeed, because of their great resistance to cold and heat, enterococci are used as indicators to identify the probable presence of enteropathogenic microorganisms, such as certain fecal coliforms (Banwart, 1979).

Enterococci occur naturally in homotherms and poikilotherms, as well as in insects and plants (Sneath et al., 1986; Diebel and Hartman, 1976). In man, these organisms can cause certain endogenous and exogenous infections. In the first case, endogenous flora can invade the organism of immunodeficient individuals, thus causing diverse health problems. Lennette et al. (1985) point out that the bacteria belonging to group D of the Lancefield classification (which also includes enterococci) are responsible for approximately 10% of all

urinary tract infections and 20% of all cases of acute bacterial endocarditis. They also note that such infections render the patient susceptible to meningitis.

In the second case, the symptoms of food poisoning caused by enterococci are mainly nausea, abdominal pains, diarrhea and, at times, vomiting (Anonymous, 1976). Humans are exposed to this type of infection because these micro-organisms commonly occur in several products destined for consumption. It should be noted that their presence in foodstuffs is rarely linked to fecal contamination (Sneath et al., 1986). The Canadian government has issued standards for the control of the quantity of enterococci in foodstuffs; Health and Welfare Canada, in its microbiological reference guide to ready-to-serve foodstuffs, has determined that the number of fecal enterococci per gram of wet or dry foodstuffs must be less than 100 (Pierre Lacasse, microbiologist, food evaluation laboratory, MAPAQ - Québec, personal communiqué, 1987).

During the air sampling program in the municipality of Manche-d'Épée, Enterococcus faecium was the only species to be detected during two consecutive days over a period of twelve days. It should be noted that the concentrations found were very low: 0.05 C.F.U./m^3 and 0.02 C.F.U./m^3, which is the equivalent of 1 and 4 C.F.U. in a 24-hour sample. It is therefore highly improbable that this minimal contamination would represent a health risk for the populations of the municipalities located in the vicinity of the areas treated under the 1987 spraying program against the spruce budworm.

BIBLIOGRAPHY

ANONYMOUS, 1976. Diseases Transmitted by Foods. U.S. Department of Health, Education and Welfare, Atlanta, Georgia 30333. 63 pp.

ANONYMOUS, 1987. Programme de pulvérisation aérienne de Bacillus thuringiensis en forêt privée dans l'est du Québec. Document prepared by l'Office des producteurs de bois de la Pocatière, the Syndicat des producteurs de bois du Bas-Saint-Laurent and the Syndicat des producteurs de bois de la Gaspésie, with the participation of the Canadian forestry service. 58 pp.

BANWART, G.J., 1979. Basic Food Microbiology. AVI Publishing Company Inc., Westport, Connecticut. 781 pp.

CARDINAL, P., 1986. Cahier des méthodologies analytiques pour le Bacillus thuringiensis. Gouvernement du Québec, ministère de l'Energie et des Ressources, Service des études environnementales. 29 pp.

COLLINS, C.H. and LYNE, M.P., 1984. Microbiological Methods. Fifth Edition, Butterworths. Chap.9.

DIEBEL, R.H. and HARTMAN, P.A. in Speck, M.L., 1976. Compendium of Methods for the Microbiological Examination of Foods. Prepared by the APHA Intersociety / Agency Committee on Microbiological Methods for Foods. 701 pp.

DOSTIE, R., MAROTTE, P.-M., DUGAL, J., CABANA, J. and DELISLE, S., 1987. Description des projets de suivi environnemental devant être effectués dans le cadre du programme de pulvérisations aériennes contre la tordeuse des bourgeons de l'épinette. Gouvernement du Québec, ministère de l'Energie et des Ressources, Service des études environnementales. 16 pp.

DUGAL, J. and MAJOR, L., 1987. Résidus d'insecticides dans l'air de certaines municipalités suite aux pulvérisations aériennes contre la tordeuse des bourgeons de l'épinette, en 1986. Gouvernement du Québec, ministère de l'Energie et des Ressources, Service des études environnementales. 29 pp.

LENNETTE, E.H., BALOWS, A., HAUSTER, W.J. and SHADOMY, H.J.,1985. Manual of Clinical Microbiology. American Society for Microbiology, Library of Congress Cataloguing in Publication Data. Fourth Edition; Washington, D.C. Chap. 16.

MAJOR, L., MAMARBACHI, G., ROUSSEAU, G., ST-LOUIS, N. and ADLEY, T., 1984a. Surveillance des pulvérisations aériennes d'insecticides contre la tordeuse des bourgeons de l'épinette, au Québec, en 1983: Echantillonage de l'air. Ministère de l'Energie et des Ressources du Québec and Eco-Recherches Inc. 30 pp.

MAJOR, L., ROUSSEAU, G. and MAMARBACHI, G., 1984b. Surveillance des pulvérisations aériennes d'insecticides contre la tordeuse des bourgeons de l'épinette, au Québec, en 1984: Résidus d'insecticides biologiques dans l'air de certaines agglomérations urbaines de la région 01. Gouvernement du Québec, ministère de l'Energie et des Ressources, Service d'entomologie et de pathologie, Division environnement et sécurité, 14 pp.

MAJOR, L., ROUSSEAU, G. and LAMONTAGNE, B., 1985. Surveillance des pulvérisations aériennes d'insecticides contre la tordeuse des bourgeons de l'épinette, au Québec, en 1984: Résidus d'insecticides biologiques dans l'air de deux agglomérations urbaines de la région 01. Gouvernement du Québec, ministère de l'Energie et des Ressources, Service d'entomologie et de pathologie, Division environnement et sécurité. 52 pp.

MAJOR, L., ROUSSEAU, G. and MAMARBACHI, G., 1986a. Résidus de fénitrothion dans l'air de trois municipalités de la région Bas-Saint-Laurent/Gaspésie. Gouvernement du Québec, ministère de l'Energie et des Ressources, Service des études environnementales. 17 pp.

MAJOR, L., ROUSSEAU, G. and CARDINAL, P., 1986b. Concentrations d'insecticides biologiques dans l'air de sept municipalités situées au voisinage des aires traitées. Gouvernement du Québec, ministère de l'Energie et des Ressources, Service des études environnementales. 33 pp.

SMIRNOFF, W.A., 1962. "A Staining Method for Differentiating Spores, Crystals and Cells of Bacillus thuringiensis (Berliner)". J. Ins. Path. 4: 384-386.

SNEATH, P.H.A., MAIR, N.S., SHARPE, M.E. and HOLT, J.G., 1986. Bergey's Manual of Systematic Bacteriology. Williams and Wilfins, Baltimore, Vol. 2, Section 12.

APPENDIX 1

Spraying operations carried out by the MER in 1987

DATE	AREA TREATED	DATE	AREA TREATED	DATE	AREA TREATED
05-31 pm	109 p[1]	06-08 am	106 p	06-13 pm	104 p
			111 p		106 p
06-01 pm	109 p		211 c[2]		108 c
			213 p		205 p
06-02 am	109 p		309 c		213 p
			310 c		306 p
06-03 pm	302 c		402 c		405 p
	303 c				
		06-08 pm	202 p	06-14 am	113 p
06-05 pm	301 p		301 p		114 c
			406 p		115 c
06-06 pm	208 p				205 p
	105 p	06-09 pm	202 p		305 p
	111 p		405 p		306 p
	308 c				405 p
		06-10 pm	106 p		
06-07 am	204 p		107 c	06-17 pm	113 p
	105 p		204 p		205 p
	106 p		208 p		206 p
	109 p		209 c		405 p
	111 p		213 p		
	304 p			06-18 am	116 p
	407 c	06-11 pm	405 p		210 c
					212 c
06-07 pm	204 p	06-13 am	104 p		405 p
	208 p		110 c		
	111 p		307 c	06-18 pm	112 p
	304 p				116 p
	401 c				201 c
					403 p
				06-19 pm	403 p
					404 c

1 p: partial
2 c: complete

APPENDIX 2

Composition of Nutrient Agar, Tween 80 and M. Enterococcus selective culture medium

- Nutrient Agar

	Per litre of distilled water
• Beef extract	3.0 g
• Peptone	5.0 g
• Agar	15.0 g

- Tween 80

  • Polyoxyethylene sorbitan mono-oleate 0.1% at pH 7.2

- M. Enterococcus

• Trypton	20 g
• Yeast extract	5 g
• Dextrose	2 g
• Phosphate dipotassium	4 g
• Sodium azide	0.4 g
• Agar	10 g
• 2, 3, 5 - triphenyltetrazolium	0.1 g
• Chloride	0.1 g
• Distilled water	1 000 ml

  • Final pH 7.2 ± 0.2 at 25°C

Gouvernement du Québec
Ministère de l'Énergie et des Ressources
Service des études environnementales